EFFECT OF DOG BREED 'VARIETIES' ON POPULATION GENETIC STRUCTURE

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INTRODUCTION

Breeding for specific characteristics in purebred dogs has resulted in a wide diversity of breeds. These breeding practices probably contributed to a reduced genetic variation within breeds. The division of dogs into different breeds is mainly based on morphological appearance and/or behaviour. However, homogeneity within breeds is not always the case. Some breeds are divided into varieties or subpopulations, based on colour types and patterns, which is achieved by reproductive isolation. The reproductive isolation provided by this “breed-fragmentation” is likely to promote genetic distance between varieties or subpopulations (Mellanby et al., 2013). When a population is divided into subpopulations, there is also less heterozygosity compared to an undivided population (Lacy, 1987).

The effects of the creation of these subpopulations on population structure and diversity have not been studied yet in Belgian breeds. Therefore we decided to investigate genetic relationships among a number of breed varieties in two Belgian dog populations, the Griffon-population and the Belgian Shepherd population.

MATERIAL AND METHODS

Animals

We examined two Belgian dog populations: 1) the Griffon population: currently managed as 3 different breeds (Griffon Belge (n= 19), Griffon Bruxellois (n= 27) and Petit Brabançon (n=55)), but crosses between these breeds are encouraged. 2) The Belgian Shepherd population, currently managed as 4 different varieties of 1 breed (Groenendael Shepherd (n= 151), Malinois Shepherd (n=1185), Tervueren Shepherd (n= 378) and Laekenois Shepherd (n=48)).

For all animals, genotyping results of 19 autosomal microsatellite markers of the 2005 International Society for Animal Genetics (ISAG) canine panel for parentage verification were available from the Belgian dog studbook "Koninklijke Maatschappij Sint-Hubertus" (KMSH). These animals were bred according to strict FCI (Fédération Cynologique Internationale) regulations. Data were obtained and were used to perform further analysis.

Analysis

Genetic divergence among populations or varieties is usually quantified by two different measures: statistics of genetic distance and Wright's fixation indices or F-statistics, FIS, FIT and FST (Wright, 1950).

FIS and FIT statistics measure the excess or the deficit of the average heterozygosity in each subpopulation and in the population as a whole respectively. The FST statistic measures the degree of genetic differentiation among populations. These fixation indices were estimated using the GENEPOP software (Raymond and Rousset, 1995) and all pairwise FST values between the subpopulations were calculated.

Furthermore, pairwise Nei's genetic distances (DS) (Nei, 1978) were computed between the varieties or breeds within the 2 populations using the

GENETIX software (Belkhir et al., 2004). The pairwise distances and FST values were then used to construct an unrooted neighbour-joining (N-J) tree using the MEGA6 software (Tamura et al., 2013).

Finally, the genetic structure of the populations was assessed using Bayesian clustering methods. The STRUCTURE software (Pritchard et al. 2000) was used to identify the number of clusters into which the 2 Belgian populations are divided. We subdivided the dogs into an increasing number of different clusters (K= 1-7). For each value of K, 4 independent runs were performed with a burn-in length of 50 000 and a run length of 500 000 Markov Chain Monte Carle (MCMC) repetitions. The program was run allowing animals to have mixed ancestry. We determined the ideal number of existing clusters, and estimated the most likely number of subpopulations K, using the STRUCTURE HARVESTER program (Earl and vonHoldt, 2012). After identifying the ideal number of clusters, analyses were repeated for the specific K-value, with more MCMC repetitions and more iterations.

RESULTS

F-statistics

Results for the estimation of the F-statistics across the populations are displayed in table 1, whereas results for the pairwise comparisons among subpopulations (or varieties) can be found in table 2 and table 3 for the Griffons and the Belgian Shepherds respectively (above the diagonal).

Table 1. Summary of Wright's F-statistics for both the Griffon and the Belgian Shepherd population.

	Griffon population	Belgian Shepherd population
FIT	0.031	0.156
FIS	-0.030	0.031
FST	0.060	0.129

FIT, or the overall inbreeding coefficient, is the reduction in heterozygosity due to non-random mating and population subdivision relative to the total population. It reaches 3.1% (FIT= 0.031) in the Griffon population, and 15.6% (FIT=0.156) in the Belgian Shepherd population.

The average genetic differentiation (FST) among varieties within the Griffon population is 0.060, which means that 6% of the total genetic diversity can be explained by population substructuring. Pairwise values between the varieties are ranging from 0.019 to 0.085. Among varieties of the Belgian Shepherd populations, the average FST value reaches 12.9% (FST=0.129), with pairwise values ranging from 0.029 to 0.275.

The within population inbreeding coefficient (FIS) reached -0.030 for the Griffon population, which indicates a (slight) excess of heterozygosity, and 0.031 for the Belgian Shepherd population, which indicates a (slight) heterozygote deficiency.

Genetic distance

As shown in table 2 (below diagonal), the Nei's pairwise genetic distances between the three varieties in the Griffon population ranged from 0.020 to 0.103.

Table 2. Pairwise comparison of FST values (above diagonal) and Nei's genetic distance DS (below diagonal) between the subpopulations of the Griffon population.

	Griffon Belge	Griffon Bruxellois	Petit Brabançon
Griffon Belge	--	0.019	0.085
Griffon Bruxellois	0.020	--	0.055
Petit Brabançon	0.103	0.062	--

In the Belgian Shepherd population, the Nei's pairwise genetic distance (Table 3, below diagonal) ranged from 0.038 to 0.644.

Table 3. Pairwise comparison of FST values (above diagonal) and Nei's genetic distance DS (below diagonal) between the subpopulations of the Belgian Shepherd population.

	Laekenois	Malinois	Groenendael	Tervueren
Laekenois	--	0.128	0.274	0.256
Malinois	0.325	--	0.137	0.260
Groenendael	0.644	0.339	--	0.029
Tervueren	0.583	0.295	0.038	--

For both distance measures, an unrooted N-J tree was made for both populations. Figure 1A indicates the N-J tree based on Nei's genetic distance for the Griffon population. The N-J tree based on FST values was very similar (not shown). We can see that the Griffon Bruxellois and Griffon Belge subpopulations are grouped together, and that they are both separated from the Petit Brabançon subpopulation.

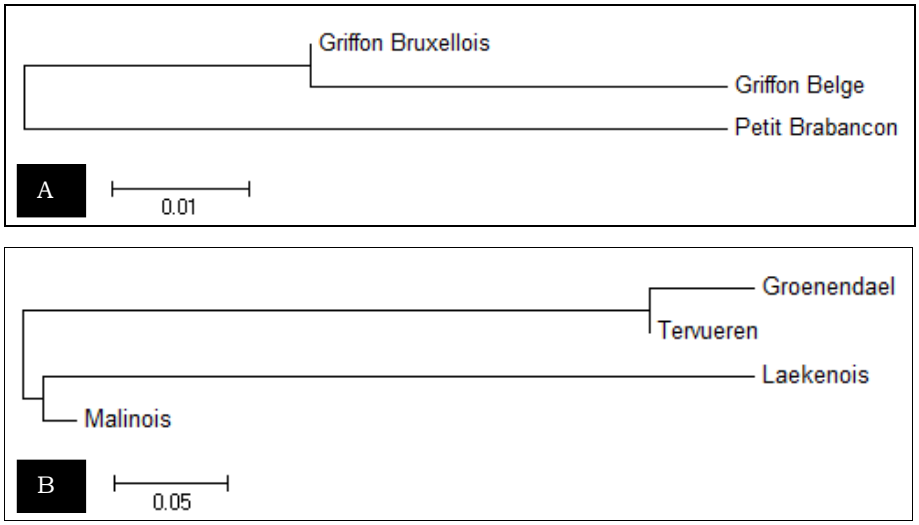

Figure 1. Neighbour-Joining tree showing the genetic relationships in the Griffon population (A) and the Belgian Shepherd population (B), based on Nei's genetic distance, calculated using the 19 microsatellites. The scale indicates the genetic distance.

Figure 1B indicates the N-J tree based on Nei's genetic distance for the Belgian Shepherd population. Here we can see that the Groenendael and Tervueren subpopulations on one hand, and the Laekenois and Malinois subpopulations on the other hand are grouped together. Furthermore, the

genetic distance in this population is five times larger compared to the Griffon population (scale difference).

Population structure

The analysis with STRUCTURE HARVESTER suggested that the likelihood of the clustering within Griffons was highest at K=2 clusters. Figure 1 shows the results of the clustering assignment of 101 dogs into two clusters. We can see that Griffon Belge and Griffon Bruxellois are clustered more or less in one cluster (assignment percentages ranging from 83%-91%), while the Petit Brabançon subpopulation constituted the other cluster. The assignment percentage of the Petit Brabançon was only 67%, which means that 33% of the animals are clustered within the Griffon Belge-Griffon Bruxellois cluster.

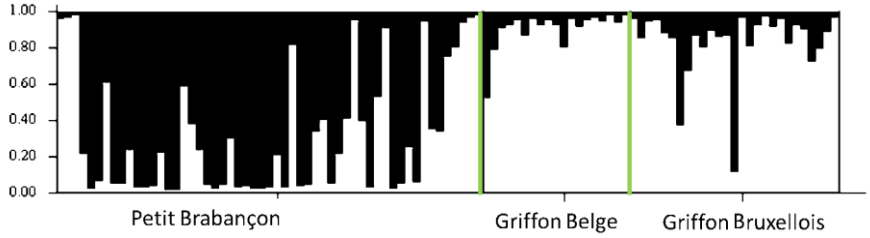

Figure 3. Clustering assignment of 101 Griffons into K=2 clusters. Each individual is represented by a vertical bar, divided into K colours. Each colour represents one cluster, and the length of the coloured segment corresponds to the individual's estimated proportion of membership in that cluster.

For the Belgian shepherds, the analysis suggested that the likelihood of the clustering was also highest at K=2 clusters. Figure 2 shows the results of the clustering assignment of the 1762 dogs into two clusters. We can see that the Malinois shepherd was clustered together with the Laekenois (assignment percentages varying from 98%-99%), while the Groenendael shepherd was clustered together with the Tervueren. The assignment percentages of these last two subpopulations ranged from 88-89%, which means that 11-12% of the animals were clustered within the Laekenois-Malinois cluster (=the white lines in the black cluster).

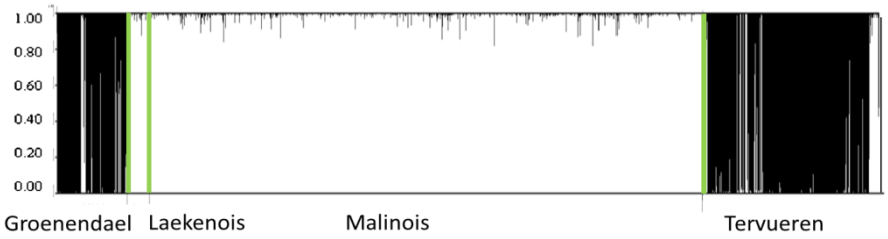

Figure 4. Clustering assignment of 1762 Belgian Shepherds into K=2 clusters. Each individual is represented by a vertical bar, divided into K colours. Each colour represents one cluster, and the length of the coloured segment corresponds to the individual's estimated proportion of membership in that cluster.

DISCUSSION

Griffon-population

Both Nei's genetic distance and the low pairwise FST values indicate that the Griffon (sub)populations are genetically very close to each other. Also the low

global FST values states this: only 6% percent of the total genetic variation is due to breed formation.

The N-J trees based on Nei's genetic distance, as well as the pairwise FST values, indicate that the Griffon Bruxellois and the Griffon Belge are grouped together, while the Petit Brabançon is genetically further away. This is also confirmed by the STRUCTURE analysis: Griffon Belge and Griffon Bruxellois are clustered together in one cluster, while the Petit Brabançon is part of the other cluster. However, more than 30% of the Petit Brabançon is wrongly assigned by STRUCTURE.

A possible explanation for the low genetic difference between the varieties is the fact that these breeds originated from one and the same breed, the "Smousje" (Vanbutsele, 2011). Furthermore, the recent encouragement of the Fédération Cynologique Internationale (FCI) regarding crosses of breeds and breed varieties may also have contributed to this low differentiation (FCI circular, 04/2012). This advice could maybe also explain the negative FIS value that was found, which indicates mating among animals having different genotypes. However, we also have to take into account the effect of a limited population size and sampling effect (few animals per population, and recent genotyping results) on which these analysis were performed. It is quite possible that the results are biased due to small sample size and more genotyped individuals per populations could therefore give a slight shift in the results.

Belgian Shepherd population

The genetic distances within the Belgian Shepherd population were higher compared to the Griffon population, and indicate that the varieties of the Belgian Shepherd are genetically further away from each other. The FIT value shows that there is a moderate reduction of the heterozygosity, probably due to non-random mating and subpopulation structure. This is also seen in the moderate FST value, which indicates the reduction of heterozygosity due to subpopulation structure. The positive FIS value illustrates that there is some effect of mating amongst relatives.

The N-J trees based on Nei's genetic distance as well as the pairwise FST values indicate that the Laekenois and Malinois shepherd are grouped together, as well as the Groenendael and Tervueren shepherd. This is also confirmed in the STRUCTURE analysis: both varieties were grouped together.

At one point in the history of the Belgian Shepherd breed (in 1898), there were only 3 varieties recognised, based on the coat texture: rough hair (Laekenois variety), short hair (Malinois variety) and long hair (both Groenendael and Tervueren varieties) (Vanbutsele, 2012). This separation can be found in our analysis, since the Groenendael and Tervueren varieties are grouped together in all analyses, and the genetic distance between these two varieties was ten times smaller than between the other varieties (e.g. 0.038 versus 0.295-0.644).

A similar study was performed within 164 poodles in Sweden by Björnerfeldt et al. (2008). It was shown that the standard poodles were very well separated from the smaller poodles (with FST-values ranging from 0.184 to 0.234, and separation confirmed with STRUCTURE analysis), which can be compared to the separation of the Laekenois, Malinois and Groenendael/Tervueren Shepherds. On the other hand, the differentiation within the group of smaller poodles (medium sized poodles, miniature poodles and toy poodles) was much lower (FST-values of -0.001 to 0.027;

STRUCTURE analysis failed to separate these breeds), similar to the Griffon population and the Groenendael-Tervueren.

CONCLUSION

The population structure observed within the Griffon population was low, possibly explained by the common ancestry of the breeds. In the Belgian Shepherd population, our analyses revealed a strong population structure, with differences among some varieties probably resulting from assortative mating imposed by breed standards as well as breeder preferences.

This study confirms that analysis based on molecular data, such as microsatellite markers, can provide a useful tool to study population structure and genetic differentiation. Results can assist organisations in the management of breeds, especially for those at risk. This may for instance be of interest in breeds with compromising genetic diversity.

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